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Research Paper

VSL#3 Probiotic Treatment Reduces Chemotherapy-Induced Diarrhea and Weight Loss

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ABSTRACT

Background: One of the most common toxicities of cancer treatment is diarrhea. Probiotics have been shown effective at preventing diarrhea in inflammatory bowel disease and may prove useful in the oncology setting.

Aim: The primary aim of this study was to investigate the probiotic mixture, VSL#3, for amelioration of chemotherapy-induced diarrhea (CID).

Methods: This experiment was carried out in a clinically relevant model of CID. VSL#3 was administered to female DA rats in one of three schedules. Irinotecan was used to induce mucositis and diarrhea, with rats monitored for seven days to record incidence of weight-loss and diarrhea. At study completion, intestines were collected to investigate histological and proliferative changes, apoptosis levels and mucin composition.

Results: VSL#3 reduced weight loss following irinotecan when administered before and after chemotherapy. Moderate and severe diarrhea was also prevented in these rats. This was associated with a significant increase in crypt proliferation combined with an inhibition of apoptosis in both the small and large intestines. VSL#3 also prevented irinotecan-induced increases in goblet cells within jejunal crypts.

Conclusions: VSL#3 is effective at preventing severe diarrhea following chemotherapy with irinotecan and therefore has potential to be used clinically by cancer patients.

INTRODUCTION

One of the most common toxicities of cancer treatment is diarrhea, yet it remains an under-researched area of supportive care. Both chemotherapy and radiation can cause diarrhea, which besides worsening quality of life for cancer patients, can also lead to interruptions in treatment and significant increases in economic burden through utilisation of supportive/adjuvant measures.[1,2] Although yet to be fully characterised, chemotherapy-induced diarrhea (CID), a component of mucositis, is associated with cytotoxic agents damaging the intestine's mucosal lining, altering water absorption.[3,4] Within the colon, water follows chloride and in normal tissue both are absorbed readily from the lumen. When the crypts of the colon are damaged from chemotherapy, chloride absorption is reduced and water is released into the lumen resulting in diarrhea. There is also alterations in gut motility, with reduced transit time for bowel contents, again resulting in a decrease in water absorption. For these reasons, CID is generally classified as osmotic or secretory in nature.[3,4] The diarrhea associated with ulcerative colitis also has an osmotic component. There is a decrease in chloride and water absorption which contributes to pathogenesis within the colon.[5] As such, there appears to be considerable overlap between the mechanisms of diarrhea in these two settings.

Importantly, chemotherapy treatment also changes the composition of the native microflora within the intestine,[4,6] although this has yet to be fully characterised. Normally, the microflora is involved in a number of gut functions, including but not limited to: protection, metabolism of bilirubin, intestinal mucins, pancreatic enzymes, fatty acids, bile acids, cholesterol and steroid hormones. Other roles of gastrointestinal bacteria include nutrient processing, regulation of intestinal angiogenesis and immune functions.[4,6] An alteration in the balance of microflora can result in a harmful environment existing within the intestine. The role of intestinal microflora in diarrhea has been high-lighted recently, through investigations into the chemotherapeutic agent, irinotecan (CPT-11), which causes severe diarrhea in the clinic. Irinotecan is converted to its highly toxic metabolite, SN38, by bacterial β-glucuronidase found in the intestine, indicating that diarrhea induced by this agent is associated with drug conversion by intestinal

microflora.[7,8] Irinotecan is also known to alter goblet cells and mucin secretion.[9] The structure of mucins allows the maintenance of the normal intestinal flora, by providing attachment sites for intestinal flora and pathogenic bacteria. It has been hypothesised that alterations in goblet cell numbers is mediated by interactions between bacterial peptides and the gastrointestinal mucosa,[10] again providing a link with diarrhea.

The probiotic compound VSL#3 (VSL Pharmaceuticals, Italy) is a new high potency preparation of highly concentrated freeze-dried living bacteria. Each commercially available sachet contains 300 billion viable bacteria per gram, comprising four strains of lactobacilli (*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus*), three strains of bifidobacteria (*Bifidobacterium infantis*, *Bifidobacterium longum* and *Bifidobacterium breve*), and one strain of streptococcus (*Streptococcus salivarius* subspecies *thermophilius*). The compound has been examined previously in models of inflammatory bowel disease (IBD) and has shown to be highly effective in ameliorating pouchitis and Crohn's disease.[11] Being a "probiotic" means that VSL#3 is capable of exerting good effects on the host organism by improving the balance of intestinal flora and by ameliorating the growth of possible pathogenic microbes.[1,12] The mechanism of action appears to be through protective, trophic and anti-inflammatory effects on bowel mucosa.[13] As such it seems sensible that VSL#3 would also be effective in mucositis, specifically CID, which has a number of overlapping pathologies with IBD, including the upregulation of inflammatory mediators.[14] Therefore the aim of this study was to investigate the potential of VSL#3 as a antimucotoxic, specifically, its ability to prevent irinotecan-induced diarrhea in a rat model of mucositis.

MATERIALS AND METHODS

Ethics. The study described here was approved by the Animal Ethics Committees of The Institute of Medical and Veterinary Sciences and of The University of Adelaide and complied with the National Health and Medical Research Council (Australia) Code of Practice for Animal Care in Research and Training (2004). Due to the potentially severe nature of the diarrhea that can be induced by irinotecan, animals were monitored four times daily and given soaked chow to aid in hydration immediately following treatment, as requested by the Animal Ethics Committee.

Animals. All experiments described were conducted in female DA rats, weighing between 150 g and 170 g. Rats were individually housed under a 12 h light/dark cycle and with ad libitum access to autoclaved food and water.

Irinotecan and VSL#3 therapy. Forty eight rats were randomly divided into 8 even groups. Groups were as follows: (1) Untreated control, (2) Irinotecan only, (3) Irinotecan + probiotic (21 days pre-treatment), (4) Irinotecan + probiotic (28 days combined pre- and post-treatment), (5) Irinotecan + probiotic (seven days post treatment), (6) probiotic only (21 days), (7) probiotic only (28 days), (8) probiotic only (seven days).

Irinotecan (CPT-11) was the chemotherapeutic agent used in this study due to its proven effectiveness in inducing diarrhea in the model.[9] Irinotecan (kindly supplied by Pfizer) was freshly prepared prior to treatment and administered in a sorbitol lactic acid buffer (45 mg/ml sorbitol/0.9 mg/ml lactic acid pH 3.4), required for activation of the drug. Rats received 0.01 mg/kg subcutaneous atropine (to reduce any cholinergic reaction to irinotecan) immediately prior to administration of a single intraperitoneal dose of 225 mg/kg irinotecan on day 0. Probiotic preparation, VSL#3 (kindly supplied by VSL Pharma), was diluted in sterile water and administered by oral gavage while rats were lightly anaesthetised (1.5% Halothane in 2 L O_2) each morning for the duration of the experiment. Each rat received 1 ml of suspension containing 3.0 x 10^8 cfu/ml of the probiotic cocktail.

Clinical observations and diarrhea assessment. All animals were assessed four times daily following the administration of irinotecan. Clinical record sheets were maintained and records kept for presence of dull/ruffled coats; change in temperament (including squealing when handled and stress marks on paws and face), diarrhea, reluctance to move and weight loss. Diarrhea was recorded according to established gradings.[15] Briefly there were four grades: 0: no diarrhea; 1: mild diarrhea (staining of anus); 2: moderate diarrhea (staining over the legs); 3: severe diarrhea (staining over the legs and abdomen, often associated with continual oozing). All diarrhea assessments were conducted in a blinded fashion by three investigators (RJG, AMS, JMB).

Organ weights. The gastrointestinal tract (from the oesophagus to the rectum) was dissected out. The intestines were separated from the oesophagus and stomach and were flushed with chilled isotonic saline. The wet weights of stomach, small intestine and colon were recorded. Small (1 cm in length) samples of the small intestine (taken at 25% of the length of the small intestine from the pylorus) and colon (taken at mid colon position) were collected and placed into 10% formalin for histological examination. In addition small samples of oesophagus and stomach were fixed in 10% formalin.

Histopathological examination. Samples of jejunum and colon were embedded in paraffin and sectioned before being routinely stained with haematoxylin and eosin. Expert histopathological examination of representative sections from each group was performed by specialist veterinary pathologist, Dr. John Finnie, from the Institute of Medical and Veterinary Science, (Adelaide, Australia). This examination was performed in a blinded fashion.

Analysis of goblet cells. Alcian blue–periodic acid Schiff method (AB-PAS). Tissue sections were dewaxed, rehydrated, and immersed in Alcian Blue solution (1% Alcian blue, 3% Acetic acid) for 5 min at room temperature before being washed and incubated in 1% aqueous periodic acid for 5 min. Slides were then washed in distilled water and immersed in Schiff's reagent for 15 min. Slides were washed in running tap water for 7½ min before lightly counterstaining (15 sec) with Lillie-Mayer's haematoxylin. Sections were dehydrated and mounted. Goblet cells were counted in 20 crypts under light microscopy by an investigator blinded to treatment (SH). The appearance of mucosubstances was also recorded for each rat (SH & JMB).

High Iron Diamine method (HID). Tissue sections were dewaxed, rehydrated and immersed in Diamine solution (1.7% Ferric chloride, 0.24% N,N-dimenthyl-m-phenylenediamine dihydrochloride, 0.04% N,N-dimenthyl-p-phenylenediamine dihydrochloride) overnight at room temperature before being washed and counterstained in Alcian Blue solution. Sections were dehydrated and mounted before analysis by light microscopy. The distribution of carboxylated and sulphated mucin types was recorded for each rat (SH & JMB).

Apoptosis assessment. Formalin-fixed, paraffin-embedded jejunum and colon samples were sectioned and subjected to TUNEL (TdT-mediated dUTP neck end labelling) assay by the In Situ cell

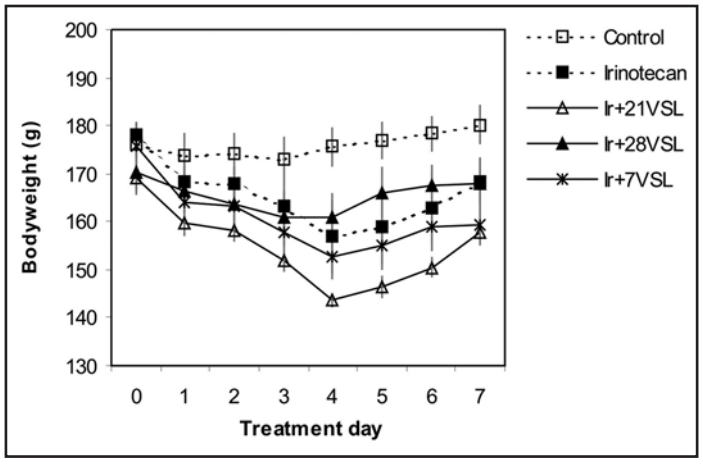

Figure 1. Graph of results for weight loss following irinotecan treatment. Administration of VSL#3 for 28 days significantly ameliorated chemotherapy-induced weight loss. Other schedules of the probiotic mixture were not effective.

death detection kit AP (Roche) according to manufacturer's instructions. The methodology employed for labelling apoptotic cells was similar to that of Gavrieli.[16] Slides were counterstained with Mayer's haematoxylin and mounted with glycerol aqueous medium. Apoptotic bodies were counted and recorded per crypt per 4 μm section in 150 crypts. This was conducted in a blinded fashion (JMB).

Crypt proliferation assessment. Proliferating cell nuclear antigen (PCNA) expression was investigated with immunohistochemistry in sections of jejunum and colon. Serial sections of tissue were cut and mounted on silane-coated slides. Sections were deparaffinised in xylene and rehydrated through a series of graded alcohols and distilled waters. Slides were immersed in 0.5% H2O2 in methanol for 10 min to block endogenous peroxidase activity. Antigen retrieval was carried out by heating slides to boiling in 0.01 M citrate buffer (pH 6.0) for 10 min. Cooled slides were immersed in Tris buffered saline (TBS, pH7.6) for 5 min before being covered with 50% horse serum in TBS for 30 min. Sections were rinsed with TBS, after which, they were incubated using the Avidin Biotin Blocking Kit (Vector Laboratories) to block endogenous biotin activity. The monoclonal PCNA antibody (Novocastra, NLC-L-PCNA) was diluted to 1:400 and applied with 5% horse serum (Sigma) in TBS. Slides were left overnight at 4°C in a humidified chamber to allow antibody reaction. Following stringent washes, the slides were incubated with a biotinylated anti-mouse secondary antibody and then streptavidin-peroxidase (Signet Laboratories, Dedham, MA). Antibody binding was visualised with 3,3'-diaminobenzidine tetrachloride (Zymed Laboratories Inc). Sections were counterstained with Mayer's haematoxylin and mounted. Positively stained cells were visualised under light microscopy. A minimum of 50 crypts per section were analysed for PCNA staining and recorded as positively stained nuclei per crypt. This was carried out blinded to treatment (JMB).

Statistical analysis. All data was grouped accordingly and expressed as the mean ± standard error of the mean. Statistical analysis of the data was carried out with the one-way ANOVA test with Tukey's post hoc test or Kruskal Wallis test and Dunn post test to identify differences between groups, using the GraphPad InStat3 package. p values <0.05 were considered statistically significant.

RESULTS

Response to treatment. Irinotecan was administered at a sub-lethal dose as described above. Animals showed adverse signs of treatment that included a dull ruffled coat, change in temperament and significant weight loss ($p < 0.05$). Weight loss peaked on day four. Rats that were treated with irinotecan alone lost 12.6% of body weight ($p < 0.05$), while rats in groups treated with irinotecan plus VSL#3 over 21 days or seven days lost 14.8% ($p < 0.01$) and 12.5% ($p < 0.05$) respectively. Treatment with irinotecan and VSL#3 for 28 days resulted in an insignificant amount of weight loss (5.3%) (Fig. 1). Rats that received no treatment or VSL#3-alone showed no signs of distress or reaction to the probiotic preparation and continued to gain weight for the duration of the trial. A total of eight unexpected deaths occurred. These were due to inhalation of the probiotic preparation passing into the trachea during gavaging. This mortality was not associated with effects of the probiotic on the gut.

Diarrhea. Irinotecan induced diarrhea in 76% of rats. In the group treated with irinotecan alone, rats developed both early and late onset diarrhea. The early diarrhea (defined as that which occurred in the first 24 h after irinotecan treatment) was mild to moderate in nature and was transient. After 48 h, the diarrhea returned, with approximately 50% of rats exhibiting mild diarrhea and 17% of rats moderate diarrhea. The peak incidence of diarrhea was 96 h after irinotecan treatment when 50% of rats developed severe diarrhea and 17% of rats developed moderate diarrhea. After this time point, diarrhea improved and was resolved completely by day seven. Rats also receiving VSL#3 in either the pre treatment or post treatment regimen followed a similar time course and severity of diarrhea except that early onset mild diarrhea persisted at 48 h. Rats given VSL#3 before and after irinotecan did not develop moderate or severe diarrhea. Early onset diarrhea was mild in nature and transient. The development of mild diarrhea after 48 h occurred in 25% of rats only and was resolved by day six (Fig. 2).

Organ weights. There was a significant increase in small intestinal weight in all groups treated with irinotecan compared to controls ($p < 0.001$). There was no difference between groups treated with VSL#3. Irinotecan treatment caused a significant increase in large intestinal weight from control weight in rats also treated with VSL#3 either pre irinotecan or post irinotecan only ($p < 0.001$ and $p < 0.05$ respectively). All other groups were not significantly altered.

Pathological reporting (inflammation). Rats in all treatment groups showed mild enterocyte hyperplasia. Also present were a few scattered apoptotic bodies in the enterocyte lining, few dilated crypts with attenuated lining epithelium and few desquamated effete enterocytes in the lumen of the colon. A mild degree of mucinous extrusion into the lumen was seen in the colon of rats treated with irinotecan alone. At the time of investigation, no evidence of inflammation was seen in either the jejunum or colon. No changes were observed in the muscle layer.

Goblet cells (mucin). Goblet cells were counted in the intestine and divided into three regions; jejunal villi, jejunal crypts and colonic crypts. No significant difference between any group was noted for goblet cell numbers in the villi or colonic crypts. Within jejunal crypts, animals treated with irinotecan alone or VSL#3 for 21 days before irinotecan had a significant increase in goblet cells ($p < 0.05$). This was prevented by treatment with VSL#3 for 28 days or seven

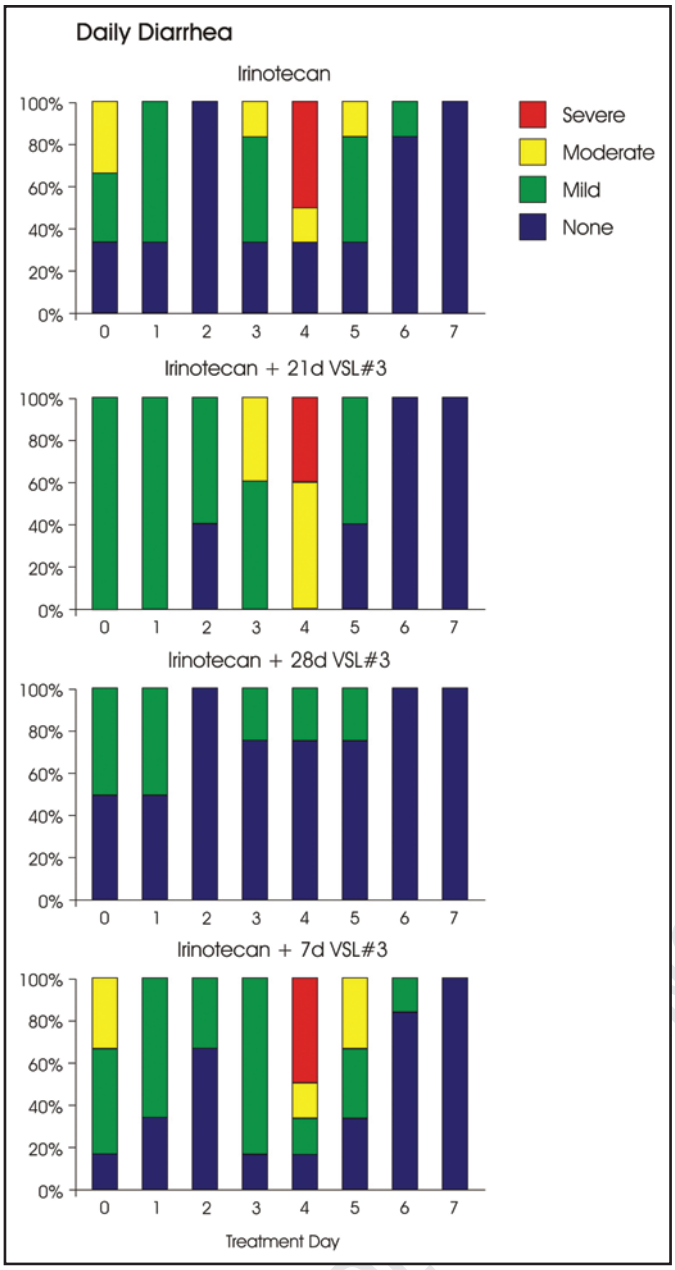

Figure 2. Results for the incidence and severity of diarrhea following irinotecan treatment. The occurrence of severe and moderate diarrhea was prevented in rats treated with VSL#3 in the combined before and after irinotecan regimen.

days following irinotecan ($p < 0.01$). Treatment with VSL#3 alone in each of the regimens did not alter goblet cell numbers (Table 1).

The distribution of acidic, mixed and neutral mucosubstances was evaluated in the small and large intestine of all rats. In the jejunum, all rats showed a predominance of mixed mucosubstances contained within globlet cells of crypts and villi, regardless of treatment. In the colon, untreated control rats showed a pattern of staining indicating a predominance of acidic mucosubstances in the basal region of crypts and a predominance of mixed mucosubstances in the apical half of crypts. This pattern was also observed in rats treated with VSL#3 for 21 days. Rats given irinotecan alone had reduced overall mucin levels within the basal region of crypts which was predominantly acidic to mixed in nature. Goblet cells in the apical region of the crypts contained mixed to neutral mucin types. Rats given irinotecan and VSL#3 for 21 days showed a similar pattern of mucins. The pattern of mucin distribution in rats treated with VSL#3 in the post irinotecan schedules was variable. Basal crypt regions showed mucosubstances that ranged from acidic to neutral with no obvious predominance. The apical portion of crypts contained goblet cells which stained mixed to neutral. This was also the pattern for rats which received VSL#3 alone in both the 28 and seven day regimen.

Mucin composition was also analysed by staining for sulphated and carboxylated mucins. The jejunal crypts and villi of all rats contained an even mixture of sulphated and carboxylated mucins irrespective of treatment. The colon showed a regionally specific pattern of mucin composition which was effected by treatment. Goblet cells within control animals exhibited a uniform pattern of mixed mucin types in the apical portion of crypts, while the basal region contained predominantly carboxylated mucin. This was altered by irinotecan treatment with an increase in sulphated mucins, especially towards the luminal region of the crypt. Treatment with VSL#3 resulted in variable changes in mucin composition. Crypts ranged from containing predominantly sulphated mucin to predominantly carboxylated or mixed between rats and also within each tissue. However, rats treated with VSL#3 for either 28 or seven days generally showed a predominance for sulphated mucins (Fig. 3).

Table 1 **Data collected for goblet cell number (GC), apoptosis (TUNEL) and proliferation (PCNA) in the rat intestine following chemotherapy and probiotic treatment**

		Control	CPT-11	CP+VSL (21d)	CP+VSL (28d)	CP+VSL (7d)	VSL (21d)	VSL (28d)	VSL (7d)
GC	Jej – v	48.9±8.76	44.5±3.95	58.8±11.6	33.2±4.77	39.7±2.07	43.2±3.71	41.5±5.99	37.6±3.48
	Jej – c	14.8±1.32	20.9±1.65*	22.3±1.4*	15.0±1.51#	16.5±1.04#	17.0±1.10	12.1±1.94	10.6±0.35
	Col	26.9±4.44	36.4±8.32	38.8±6.39	37.9±4.77	42.4±2.62	28.6±4.00	31.4±3.53	32.7±1.57
TUNEL	Jej	0.05±0.01	0.17±0.02*	0.23±0.05*	0.07±0.01#	0.13±0.03	0.12±0.03	0.08±0.03#	0.04±0.01#
	Col	0.07±0.01	0.35±0.15*	0.19±0.03	0.09±0.03#	0.18±0.02	0.12±0.02	0.13±0.04	0.05±0.01#
PCNA	Jej	10.4±1.18	7.39±0.18	9.00±0.26	16.9±2.2*#	14.7±1.45#	10.9±1.34	14.1±1.91#	13.5±0.89#
	Col	12.6±0.60	9.44±0.51	10.5±1.18	16.6±2.17#	16.1±0.54#	12.4±0.95	19.1±1.65#	17.5±0.38#

Statistical significance compared to controls, where * denotes $p < 0.05$. Statistical significance compared to CPT-11, where # denotes $p < 0.05$. Data shown is group mean ± SEM ($n = 4–6$).

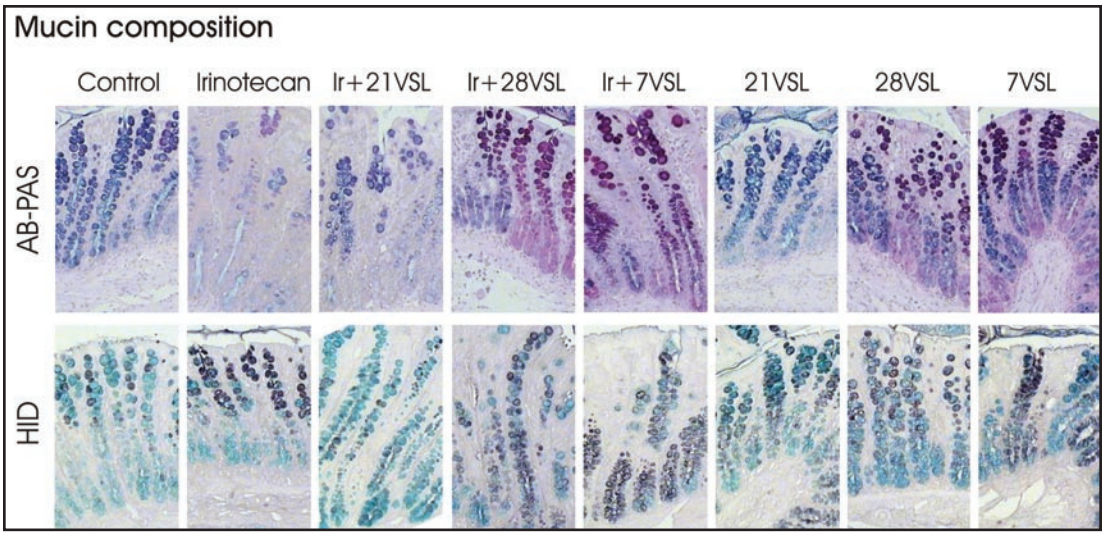

Figure 3. Distribution of mucosubstances in the rat colon following treatment with irinotecan and VSL#3. Following AB-PAS staining, acidic mucins stain dark blue, mixed stain purple and neutral stain pink in colour. The HID stain results in sulphated mucins appearing brown and carboxylated mucins appearing light blue. The effect of VSL#3 on predominance of mucin type was variable. Photomicrographs original magnification x100.

Apoptosis (protective). Apoptotic cells within intestinal crypts were labelled using the TUNEL assay. Irinotecan treatment alone caused a significant increase in crypt apoptosis in both the jejunum and colon ($p < 0.05$). Apoptosis was also significantly increased in the jejunum of rats pre-treated with 21 days of VSL#3 and irinotecan. Treatment with VSL#3 in the 28 day regimen prevented any increase in apoptosis associated with irinotecan ($p < 0.05$). Rats treated with VSL#3 alone in any of the protocols showed no alteration in apoptosis levels compared to control animals (Table 1).

PCNA (trophic). The effect of VSL#3 on proliferation was assessed via crypt PCNA expression. Rats in groups which received VSL#3 following chemotherapy had significantly increased numbers of cells positively stained for PCNA within intestinal crypts compared to controls and those treated with irinotecan alone ($p < 0.05$). This was evident in both the jejunum and colon. VSL#3 given before chemotherapy did not alter the expression of PCNA within the intestine, regardless of combination treatment with irinotecan. VSL#3 alone given for 28 days or seven days caused a significant increase in PCNA staining within the colon compared to controls ($p < 0.01$) (Fig. 4 and Table 1).

DISCUSSION

Recently, the introduction of probiotic use in controlling IBD-associated diarrhea has evolved into the cancer arena, where it may also prove useful in preventing chemotherapy treatment-induced diarrhea. This study has been the first to investigate the probiotic preparation, VSL#3, in the prevention of chemotherapy-induced diarrhea (CID) and mucositis. The model used in this study is proven to closely mimic mucositis in cancer patients.[9,15,17] We have shown that VSL#3 is effective in reducing diarrhea following irinotecan administration in a schedule-specific manner. The mechanisms by which it is effective appear to be three-fold. Firstly, we have shown that VSL#3 increases epithelial proliferation, and as such, may be involved in healing of the mucosal layer following chemotherapy treatment. Secondly, VSL#3 reduces intestinal apoptosis in response to chemotherapy treatment, therefore helping to prevent mucosal breakdown and crypt damage. Thirdly, VSL#3 prevents the increase in goblet cell number and mucin secretion which occurs following irinotecan treatment, as such helping to maintain water and electrolyte balance within the intestine, preventing onset of diarrhea. However, it is important to note that the protective effects of VSL#3 are maximal only when given in a specific regimen, namely before and after chemotherapy treatment.

VSL#3 acts as a trophic agent equally in the small and large intestine of the rat. Treated rats showed crypt hyperplasia and an increase in PCNA expression indicating an environment of active proliferation. The effect of VSL#3 was transient however, as the change in PCNA expression was not observed in rats pre-treated with VSL#3 prior to chemotherapy. This also suggests that the beneficial effects of VSL#3 are built up over the constant course of treatment, but disappear rapidly once administration stops. A potential mechanism for the protective effects of VSL#3 throughout the gut could be an increase in proliferation following irinotecan, aiding in the recovery phase of mucosal damage.

The effect of probiotics on intestinal apoptosis has previously been investigated. Linsalata et al.[18] reported that VSL#3 increases apoptosis in the distal colon of healthy rats. Our study did not support this finding, with no significant change in apoptosis in the intestines of rats treated with VSL#3 alone being noted. A possible explanation for this is the region of intestine investigated. We collected and examined sections of mid-colon. Whereas Linsalata found a decrease in apoptosis in the distal colon, they also found no significant difference in apoptosis levels in the proximal colon of rats treated with VSL#3 compared to saline controls.[18] This suggests that VSL#3 exerts a pro-apoptotic effect which is specific only to the distal colon region in normal rats. VSL#3 did ameliorate apoptosis in response to irinotecan treatment. This finding is supported by the work carried out by Yan and Polk.[19] They showed that probiotic treatment prevents intestinal epithelial cell apoptosis in response to tumour necrosis factor (TNF), interleukin-1 alpha (IL-1α) and γ interferon (IFN).[19] These pro-inflammatory factors have been implicated in the pathogenesis of chemotherapy-induced mucositis.[20-22] Proposed mechanisms for probiotic-induced protection from apoptosis include activation of the anti-apoptotic Akt/protein kinase B and also through inhibition of the pro-apoptotic p38/mitogen activated protein kinase.[19] It was interesting to note that apoptosis was reduced only in the rats which received VSL#3 both before and following irinotecan. This may indicate that VSL#3 works in two ways to prevent cell death following chemotherapy. Firstly, VSL#3 given before chemotherapy may increase the resistance to immediate damage caused by the direct toxic effects of irinotecan. And secondly, the on-going VSL#3 treatment may be important to continue this resilience

from damage induced by pro-inflammatory factors activated following irinotecan and in response to generalised tissue damage. This would in part explain why giving VSL#3 only following irinotecan did not improve apoptosis or diarrhea in this study.

Our investigations of mucin composition following chemotherapy and in response to VSL#3 found a number of changes. Firstly, irinotecan treatment caused an increase in goblet cells within jejunal crypts which was prevented by VSL#3. Previous work by our group has shown an increase in mucin secretion in response to irinotecan treatment which is associated with mucosal damage.[9] Furthermore, irinotecan altered the predominance of acid mucosubstances in the basal region of colonic crypts to a predominance of mixed mucosubtances. Additional treatment with VSL#3 resulted in variable changes to mucin staining within the colon and was considered not a primary mechanism in diarrhea induction. This was also the case for distribution of carboxylated and sulphated mucins within the colon. It was observed that irinotecan increased the proportion of sulphated mucins within crypts, however when investigated in combination with VSL#3 treatment, no consistent changes could be noted. From these results we can assume that mucin composition is not a major determinant in the pathobiology of diarrhea in response to irinotecan treatment in the current mucositis model. This does not negate the importance of probiotic treatment on maintenance and composition of the mucus layer in other models of diarrhea.

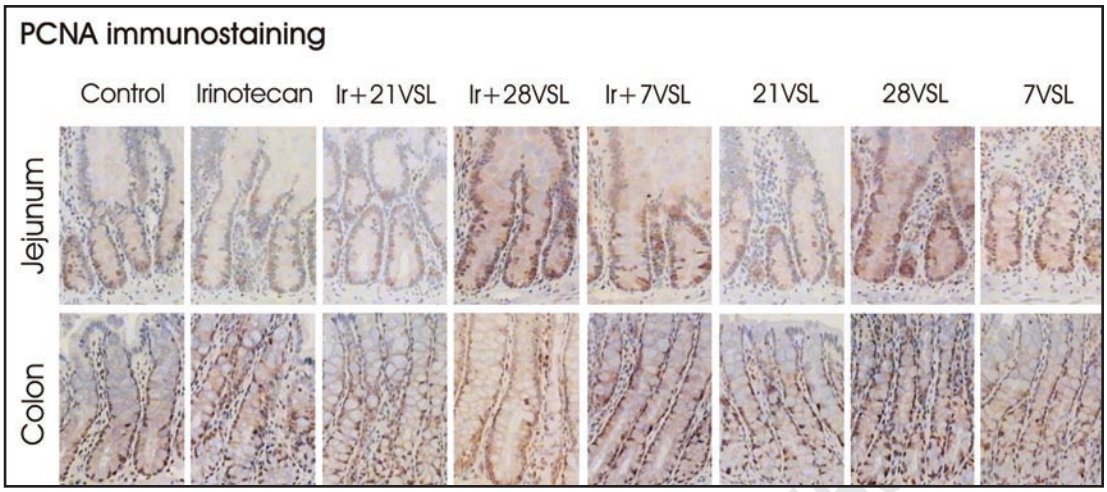

Figure 4. Investigation of proliferation in the intestine following irinotecan and VSL#3 treatment. Staining for PCNA was increased in the crypts of the jejunum and colon in rats treated with VSL#3 for 28 or seven days. Photomicrographs original magnification x100.

In conclusion, this study has found that the probiotic preparation, VSL#3, is indeed effective in ameliorating mucositis through prevention of CID. The protective effects of VSL#3 are regimen-specific and require that the agent to be administered both leading up to and following chemotherapy treatment to be most beneficial. We propose that the anti-diarrheal mechanisms include enhanced epithelial proliferation and reduced apoptosis to ameliorate crypt damage. VSL#3 also helps prevent excess mucin secretion but does not appear to alter mucin composition significantly. Further studies into the effectiveness of VSL#3 as an anti-mucotoxic are warranted.

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